APLICACIONES DE LA CITOMETRÍA DE FLUJO EN LA PRÁCTICA CLÍNICA

ASOCIACIÓN BIOQUÍMICA ARGENTINA – GRUPO RIOPLATENSE DE CITOMETRIA DE FLUJO

PATRONES INMUNOFENOTÍPICOS EN MÉDULA ÓSEA NORMAL / LINFOPOYESIS

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Utilidad de la citometría en oncohematología

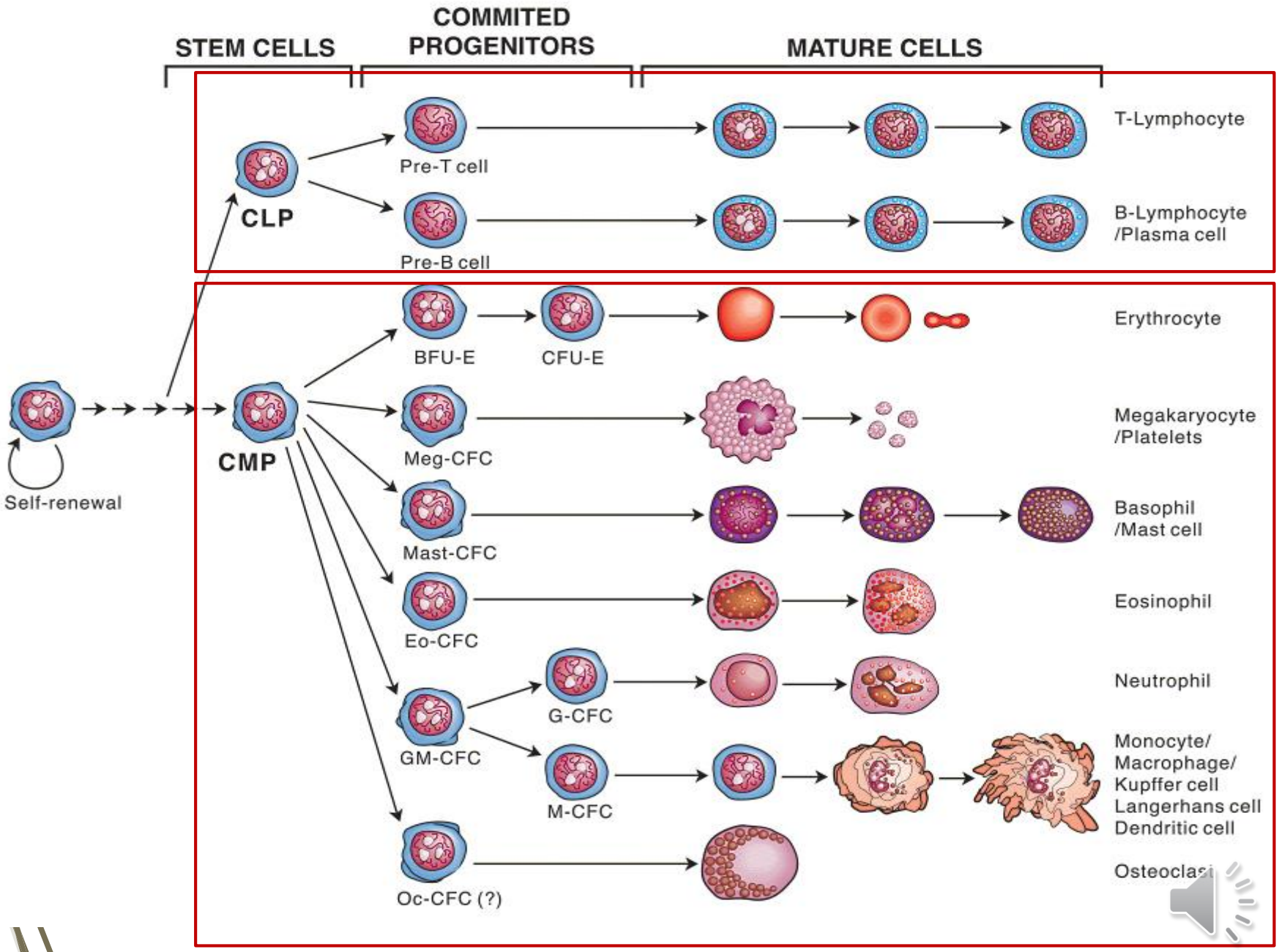
- Diagnóstico
- Clasificación pronóstica
- Evaluación de la efectividad del tratamiento

Caracterización inmunofenotípica

- ¿Cuan similar son las células leucémicas a las células normales?

- ¿Cuanto difieren las células leucémicas de las células normales?

HEMATOPOYESIS



Hematopoyesis

- **Mielopoyesis** Los progenitores de las plaquetas, hematíes, granulocitos y monocitos realizan todo su proceso de proliferación y diferenciación en la médula ósea.

- **Linfopoyesis** es la producción de linfocitos y a diferencia de los que ocurre con los demás tipos celulares de la sangre, los linfocitos también proliferan y maduran fuera de la médula ósea

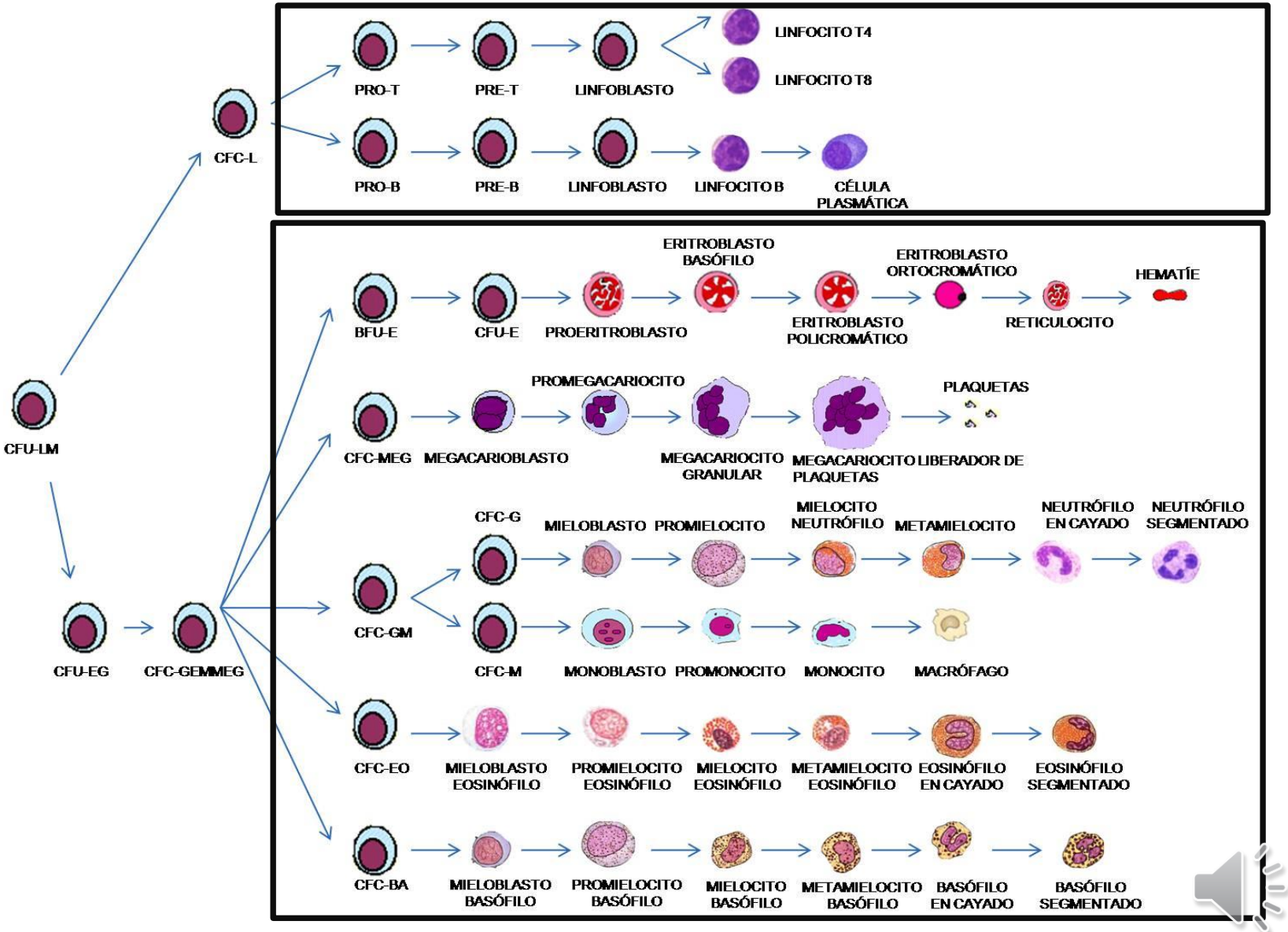
Hematopoyesis

Producción diaria: GR, GB, plaquetas

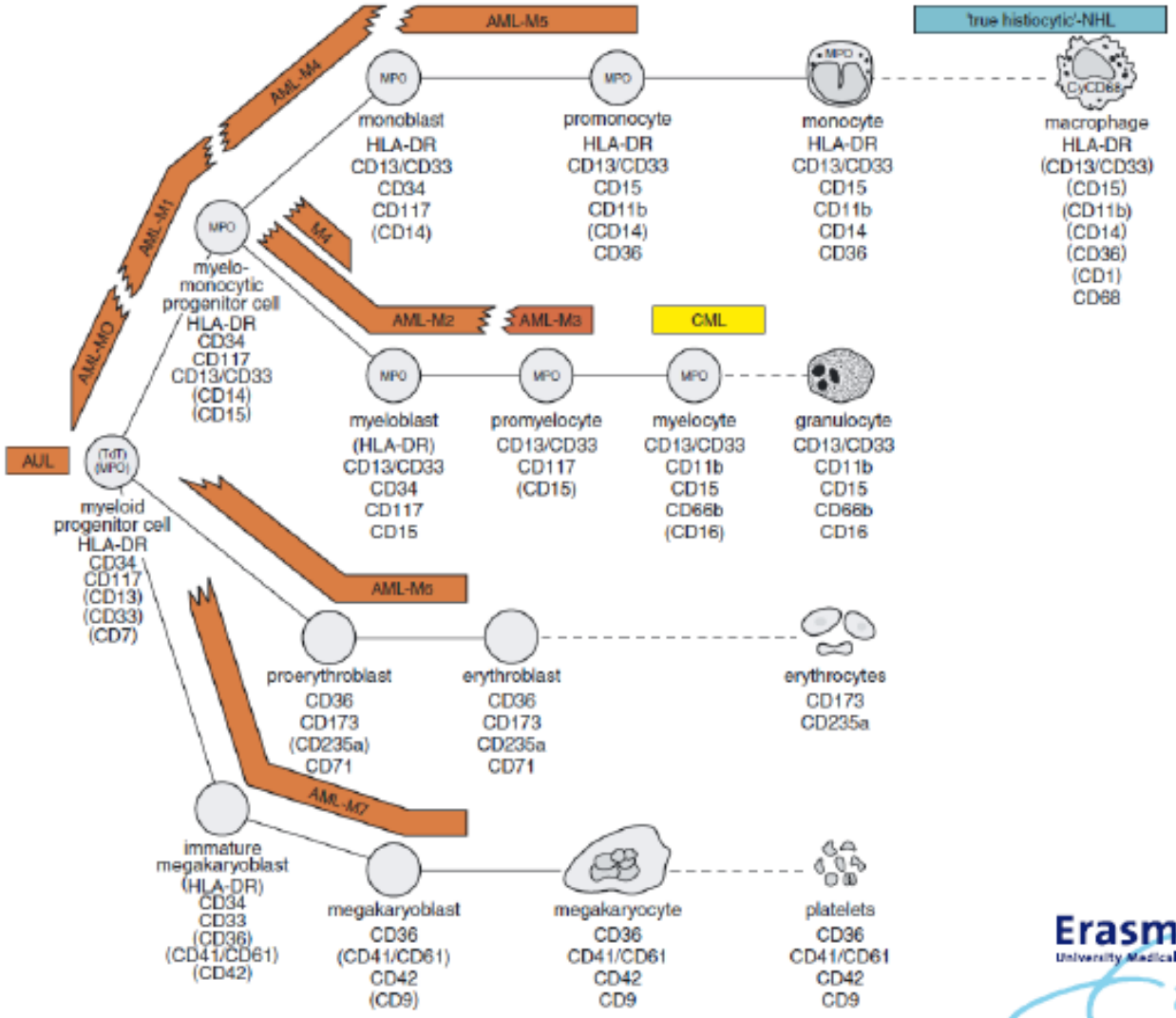
- **175 billones GR/día**
- **70 billones granulocitos/día (N,Eo,Ba)**
- **175 billones plaquetas/día**

⬆ de producción: 5-10 veces

Hematopoyesis

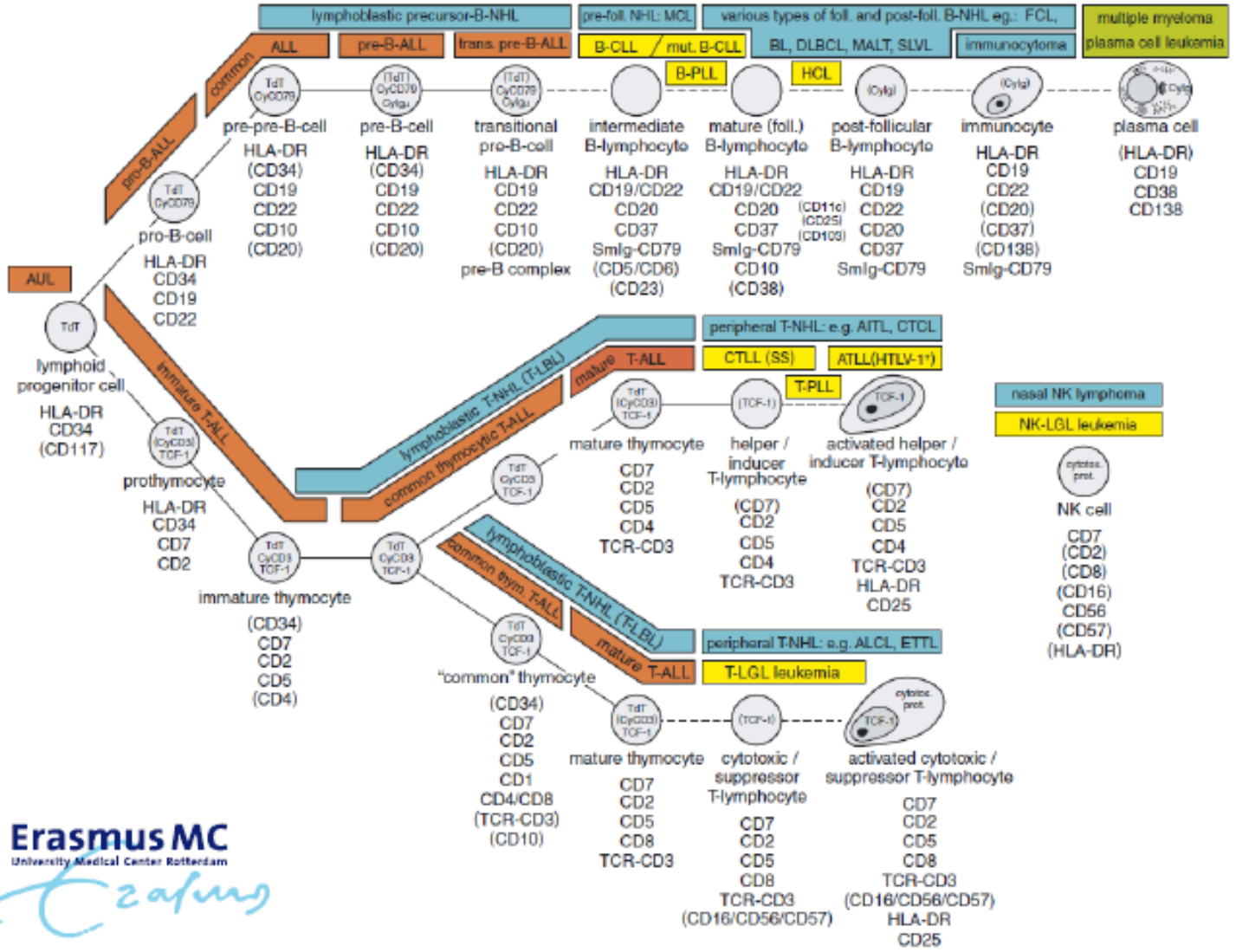


Myeloid malignancies



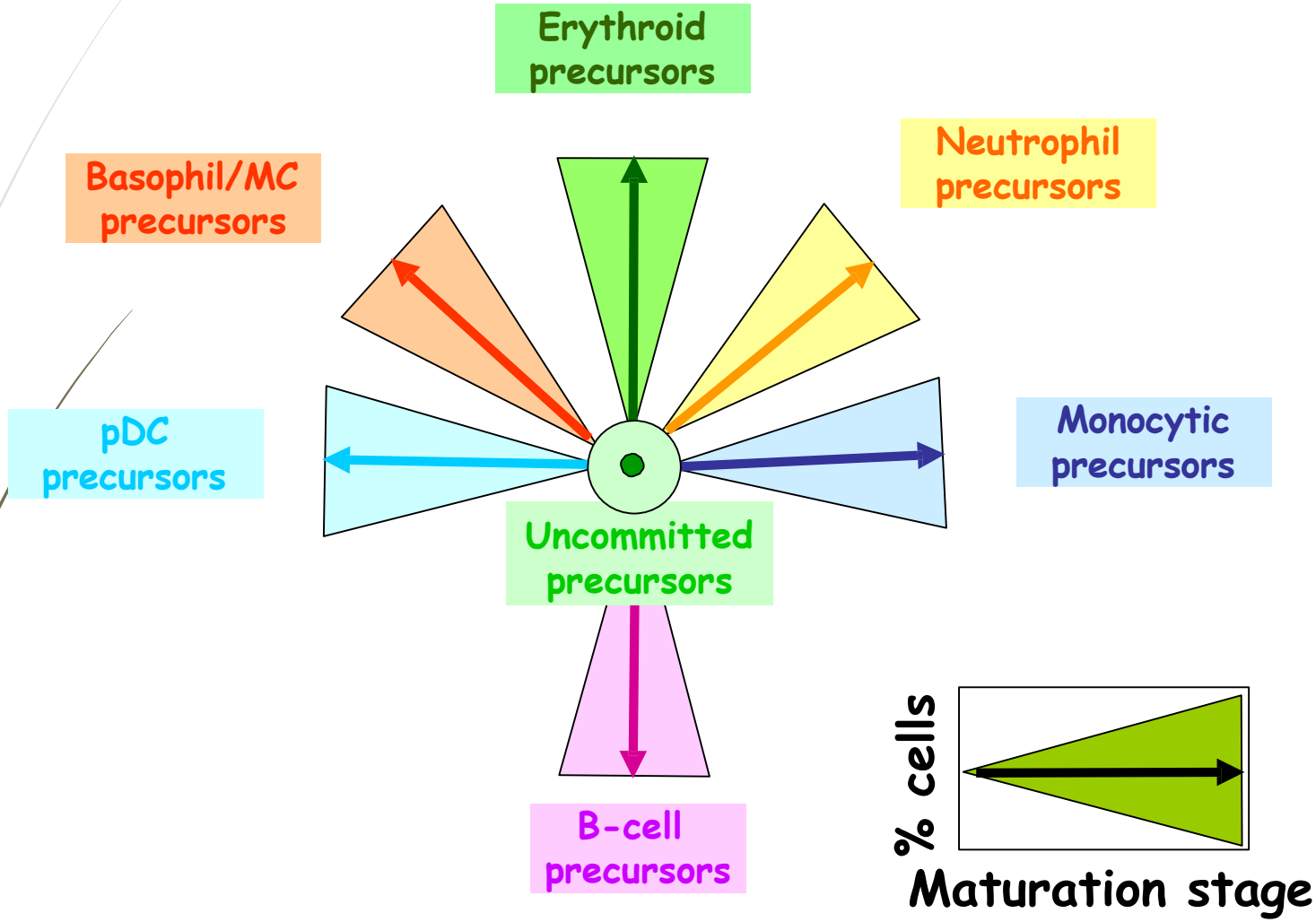
Erasmus MC
University Medical Center Rotterdam

Lymphoid malignancies



Erasmus MC
University Medical Center Rotterdam

Diferenciación de CD34+ HPC hacia diferentes líneas hematopoyéticas



CD34+HPC

CELL LINEAGE	FREQUENCY
Erythroid	15% (5%-35%)
Megakaryocytic	<1%
Neutrophil	31% (12-39%)
Eosinophil	<1%
Basophil	<1% (<1%-3%)
Monocytic	5% (3%-15%)
Mast cell	<1% (<1%)
pDC	6% (1%-15%)
B-Lymphoid	23% (<1%-45%)
CD7+ (Early T-lymphoid?)	11% (5-18%)
Total:	92%

EuroFlow

Patrones inmunofenotípicos de la diferenciación linfoide

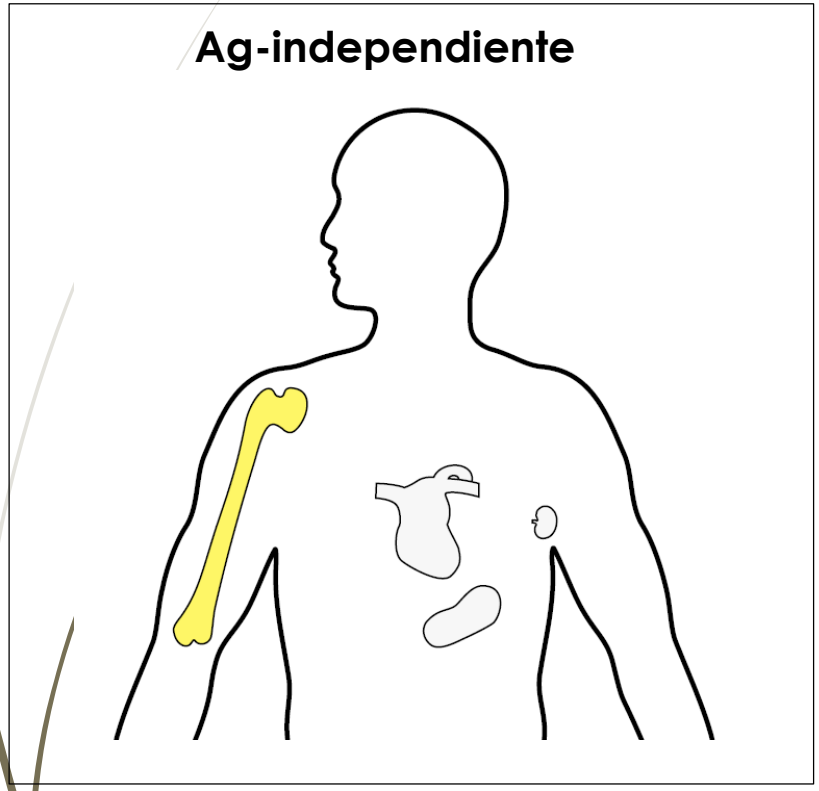
Linfopoyesis

- A partir de un mismo progenitor linfoide se generan distintas líneas celulares de maduración diferenciadas:

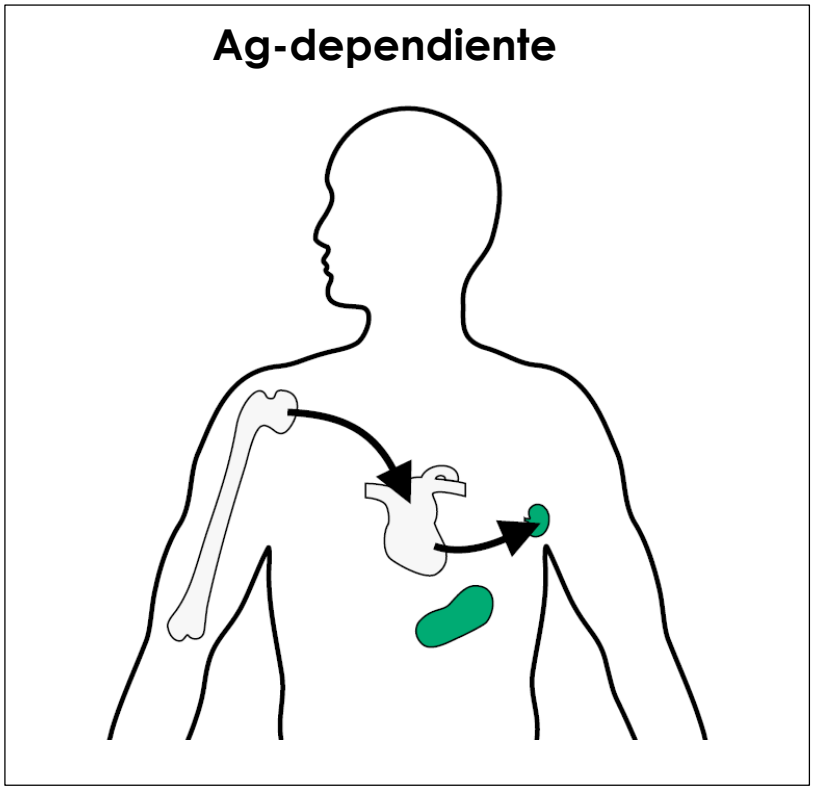
  - Linfocito B
  - Linfocito T
  - Células Natural Killer
  - Célula dendrítica plasmocitoide

DIFERENCIACIÓN LINFOIDE B

Diferenciación linfoide B

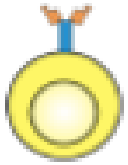


Médula ósea (adulto): Generación de linfocitos B inmunocompetentes (BCR) y autotolerantes



Migración de los linfocitos B maduros de la MO a los órganos linfoides secundarios

Diferenciación de la célula B en la MO

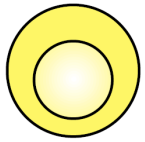
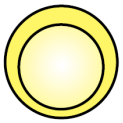
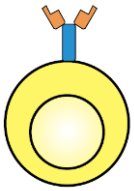
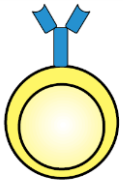
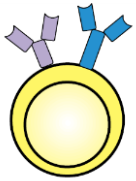
	Common Lymphoid Precusor	Early Pro-B	Late Pro-B	Pre-B	Immature B-cell	Naïve B-cell
						
IgH genes	Germinal	D-J Rearrag.	V-DJ Rearrag.	VDJ	VDJ	VDJ
IgL genes	Germinal	Germinal	Germinal	Reord. V-J	VJ	VJ
Surface IG	-	-	-	Cadena μ (pre-BCR)	IgM	IgM + IgD
Differen. factors	CAM CD44	SCF CXCL-12 IL-7	SCF IL-7	IL-7 preBCR	BCR	
Transcript. Control		Pax-5	Pax-5	Pax-5	Pax-5	Pax-5

Selección positiva

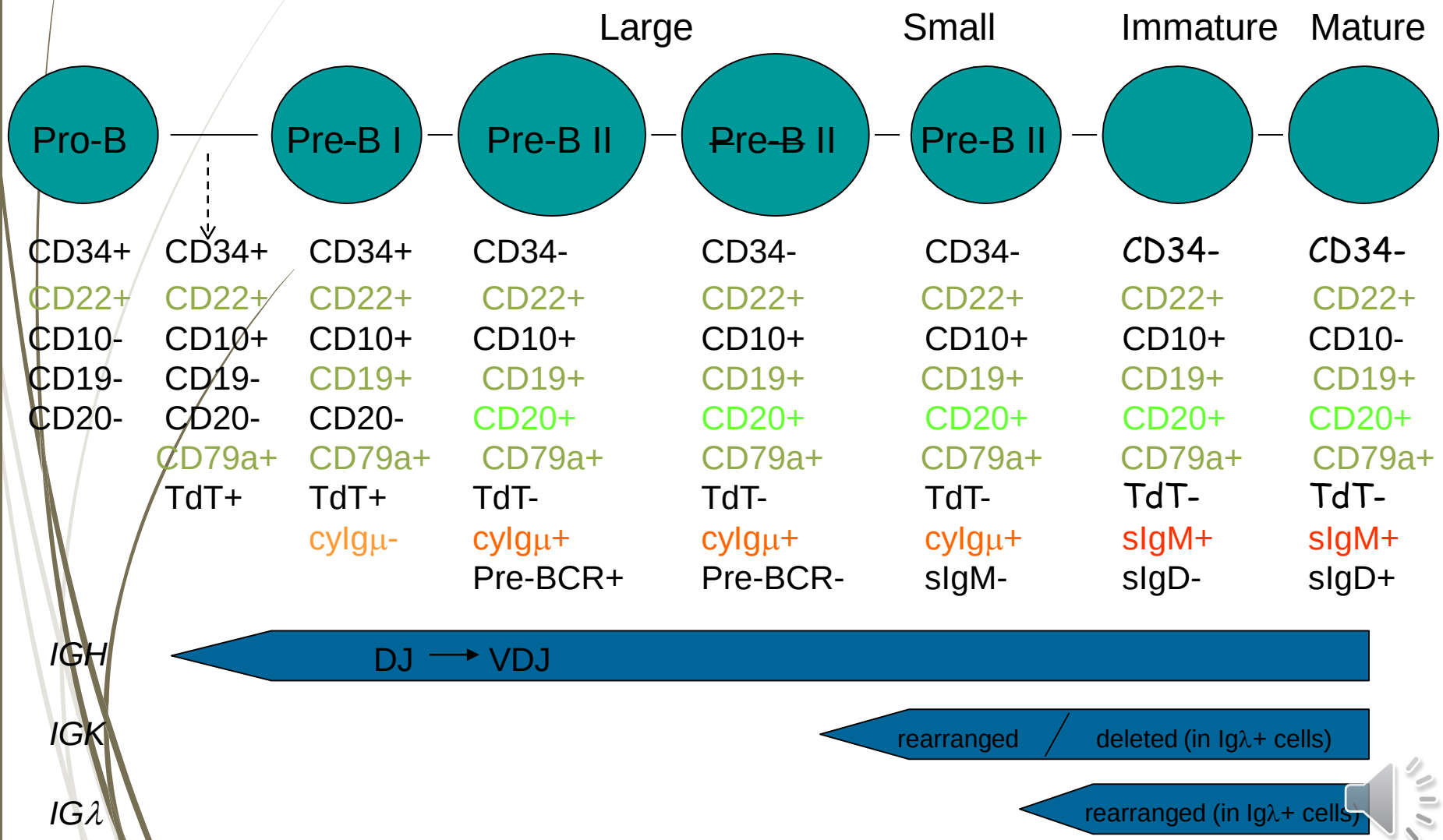
Selección negativa

Blom B. Annu. Rev. Immunol. 2006

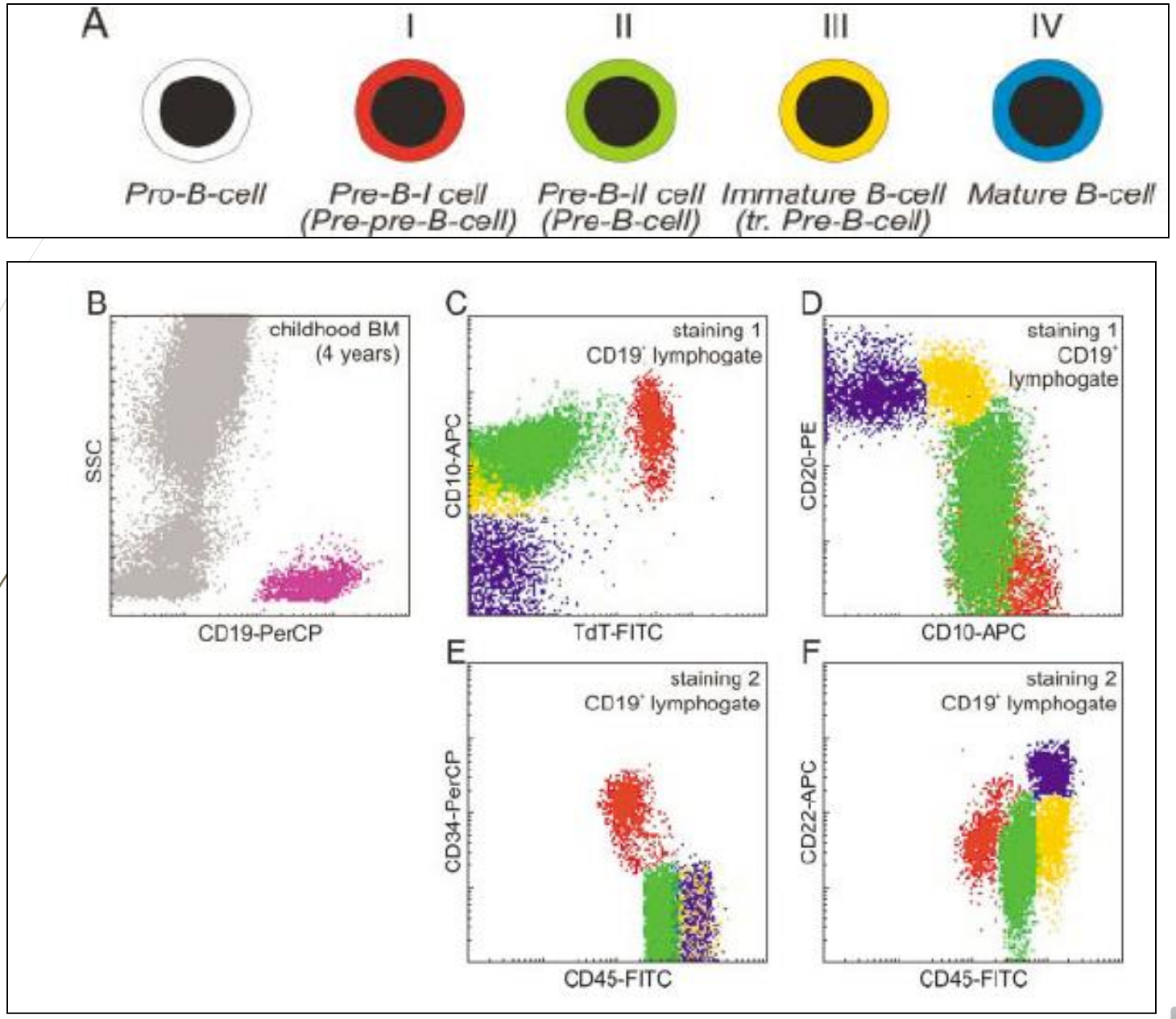
Cambios asociados a la diferenciación linfoide B antígeno-independiente

	Célula madre linfoide	Pro-B temprana	Pro-B tardía	Pre-B	Linfocito B inmaduro	Linfocito B maduro
						
Genes IgH	Germinal	Reord. D-J	Reord. V-DJ	VDJ reordenado	VDJ reordenado	VDJ reordenado
Genes IgL	Germinal	Germinal	Germinal	Reord. V-J	VJ reordenado	VJ reordenado
Ig superficie	Ausente	Ausente	Ausente	Cadena μ (pre-BCR)	IgM	IgM + IgD
Otras moléculas	**CD34** **CD19-/+** **CD22** CD38 HLA-II **TdT**	**CD10** **CD19** CD38 CD40 CD24 CD45R CD43 HLA-II **TdT**	**CD10** **CD19** CD38 CD40 CD24 CD45R CD43 HLA-II **TdT**	**CD19** CD38 CD40 CD24 CD45R CD43 CD20 HLA-II **TdT**	**CD19** CD21 CD40 CD24 CD45R CD20 HLA-II	**CD19** CD21 CD40 CD24 CD45R CD20 HLA-II

Diferenciación linfocitaria B



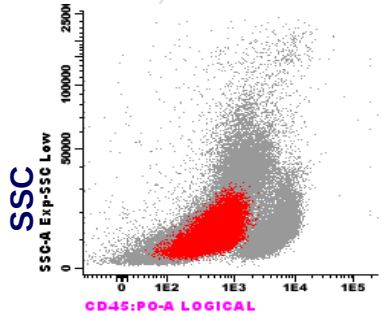
Ghia et al, 1996; Lucio et al, 1999; LeBien et al, 2000



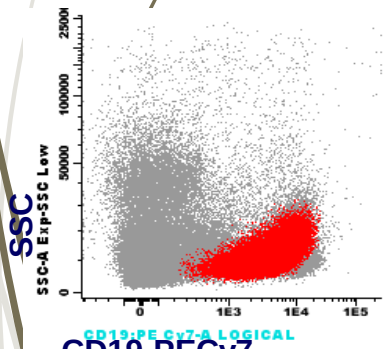
Van Lochem et al, Cytometry 2004

Inmunofenotipo de LB normales vs LB leucémicos

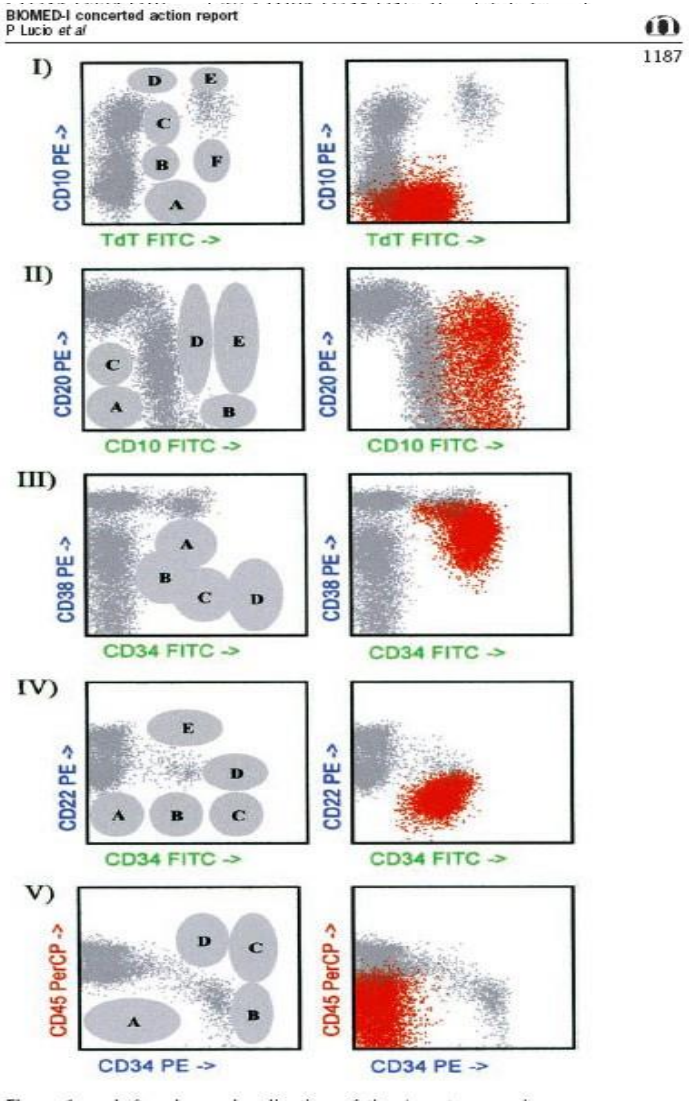
CD19+
B-CELLS



CD45-PO

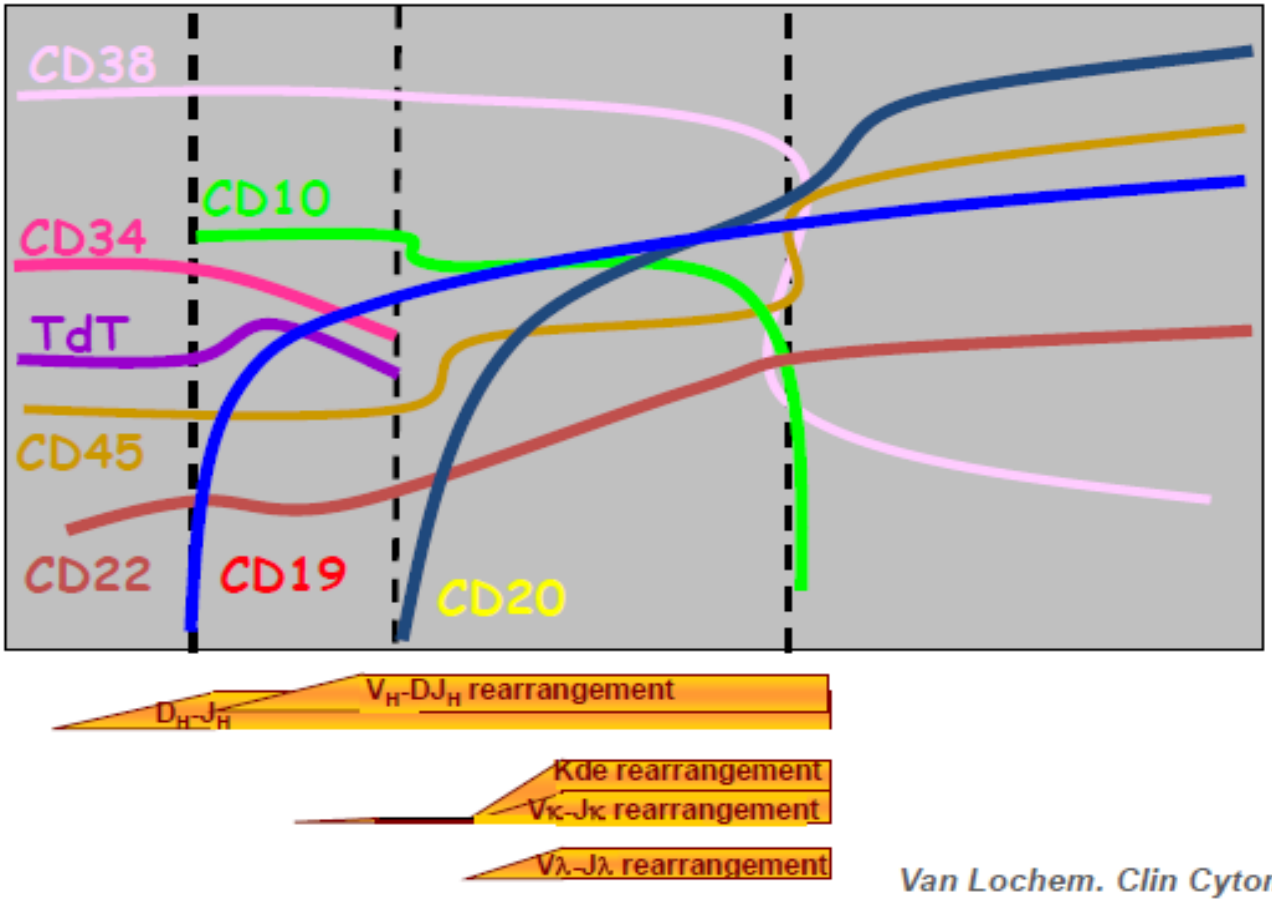


CD19-PECy7



Lucio et al, Leukemia, 1999

Perfil fenotípico de los precursores B



Van Lochem. Clin Cytom 2004

EGIL (II)

Subtipos

PRO-B/ NULA

CD10: -

cIgM: -

COMUN

CD10: +

cIgM: -

PRE-B

CD10: +; CD20 +het

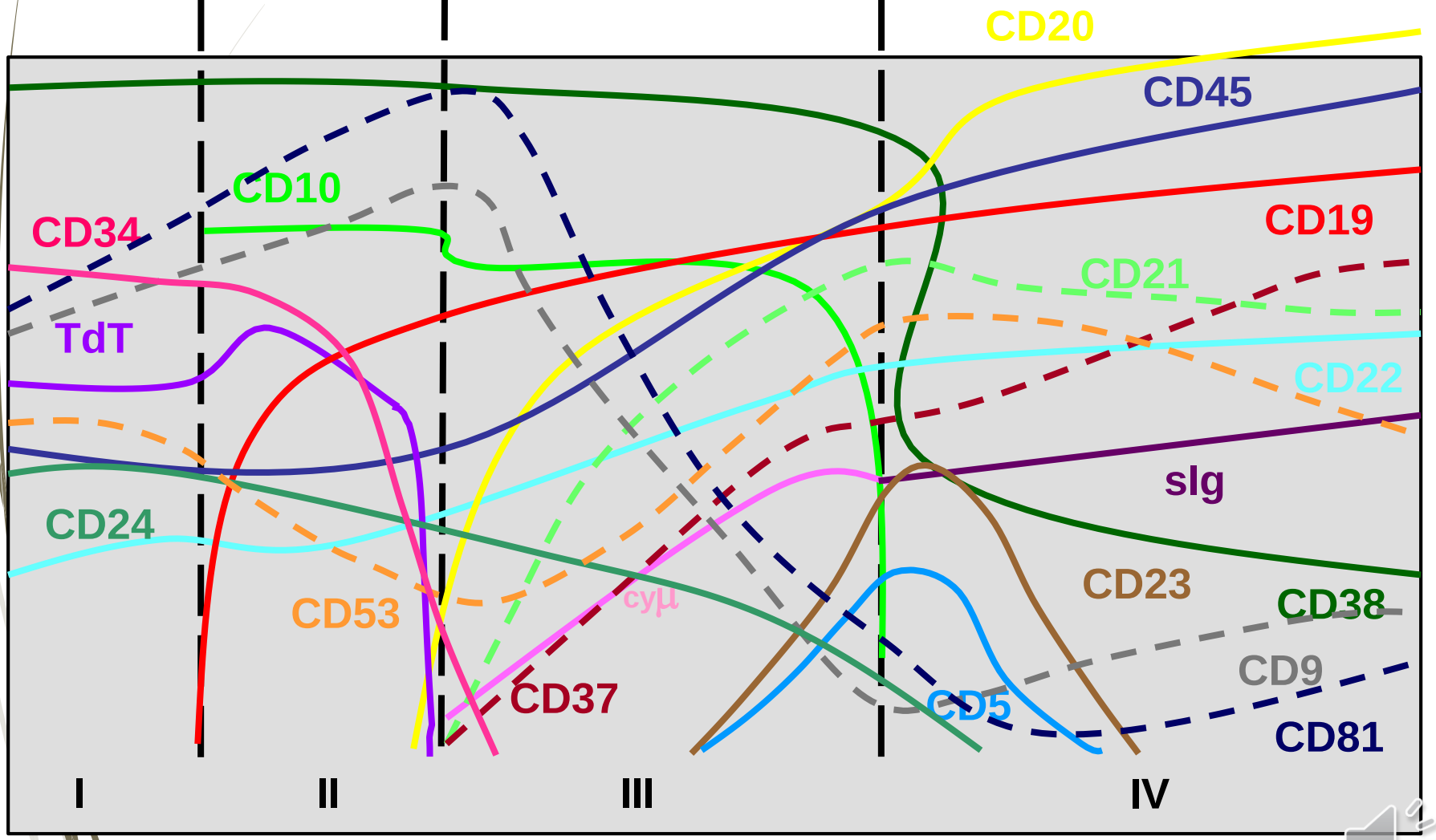
cIgM: +; sIg: -

B-ALL Maduro

CD10: -/+; CD20 +

cIgM: +; sIg: +

Diferenciación fenotípica de los precursores B



BCP ALL PANEL

EuroFlow

OBJETIVOS GENERALES:

1. IDENTIFICAR.

2. CUANTIFICAR

3. CARACTERIZAR

Marcadores relevantes para establecer PATRONES DE MADURACIÓN:
CD10-CD20; CD34-CD38; cyIgM; smIgκ smIgλ; nuTdT

Marcadores relacionados con INFIDELIDAD DE LINEA:
CD13;CD33;CD15;CD65;CD123

Marcadores relacionados con presencia de aberraciones genotípicas recurrentes:
CD9; CD10; CD66c; NG2 (7.1)

Multi-tube EuroFlow classification panel for B-cell precursor ALL (BCP-ALL)*

Responsible scientist: L Lhermitte

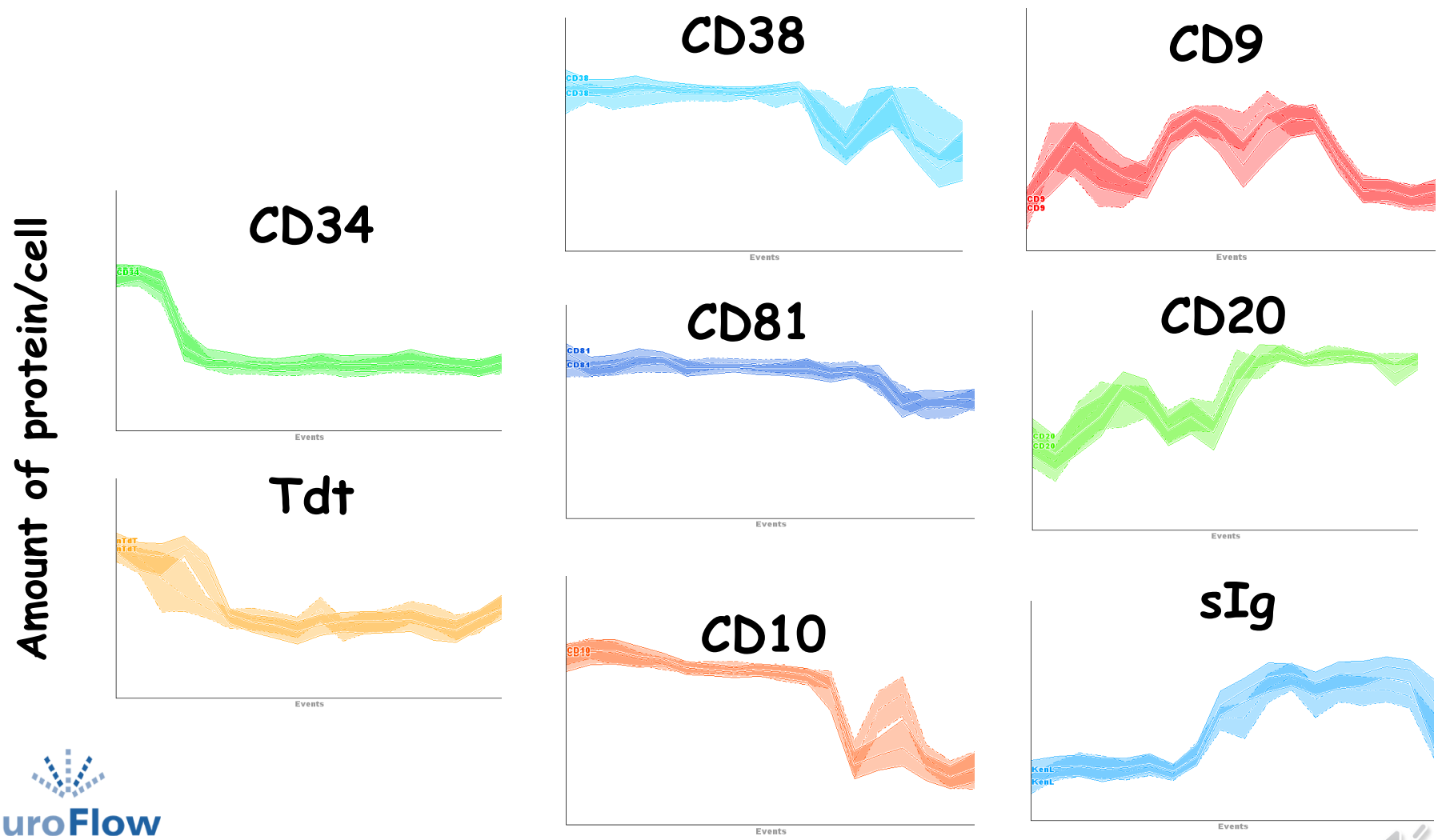
Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	CD20	**CD45**	CD58	CD66c	**CD34**	**CD19**	CD10	CD38	Diagnosis and classification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations
2	SmIgκ	**CD45**	CyIgμ	CD33	**CD34**	**CD19**	SmIgμ and CD117	SmIgλ	Diagnosis and classification of BCP-ALL;
3	CD9	**CD45**	NuTdT	CD13	**CD34**	**CD19**	CD22	CD24	Diagnosis and classification of BCP-ALL; Detection of phenotypes associated with molecular aberrations; Detection of LAP markers
4	CD21	**CD45**	CD15 and CDw65	NG2	**CD34**	**CD19**	CD123	CD81	Subclassification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations

* Backbone markers are indicated in bold; Cy= cytoplasmic; Sm= surface membrane; Nu= nuclear.

** The described marker combinations can also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics).

EuroFlow

NORMAL B-CELL MATURATION IN BM
Protein expression profiles



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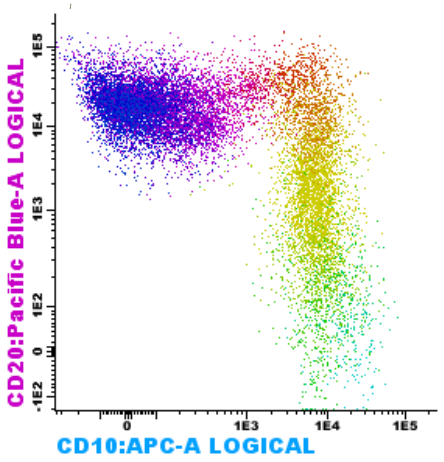
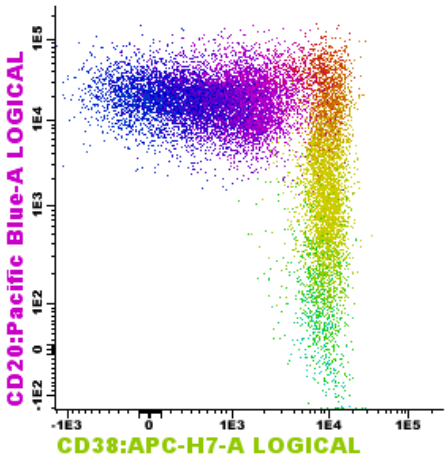
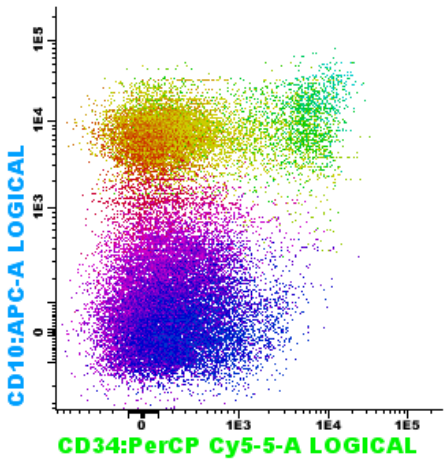
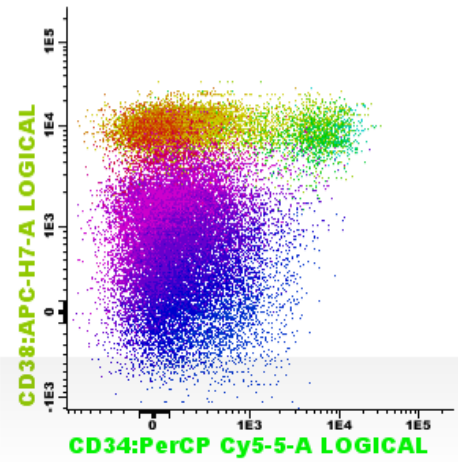
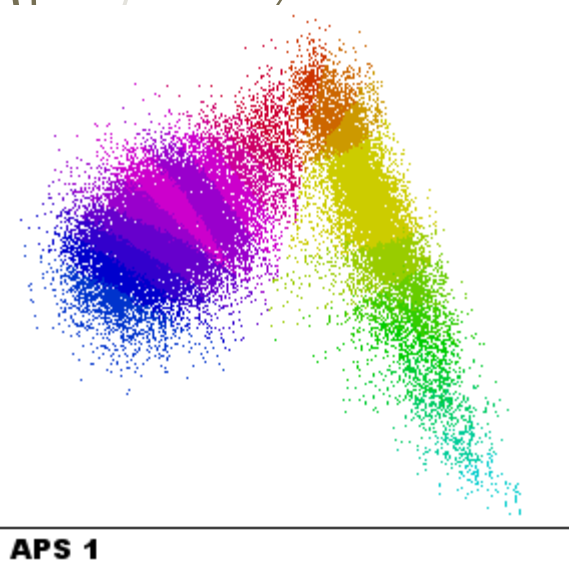
Disección automática de la diferenciación de línea B











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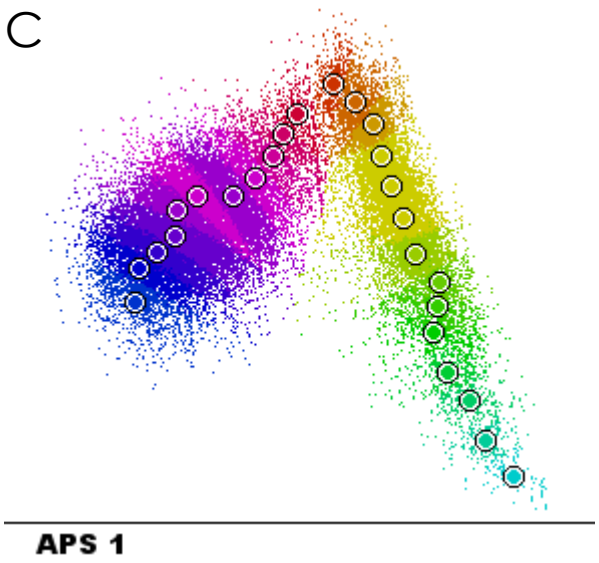
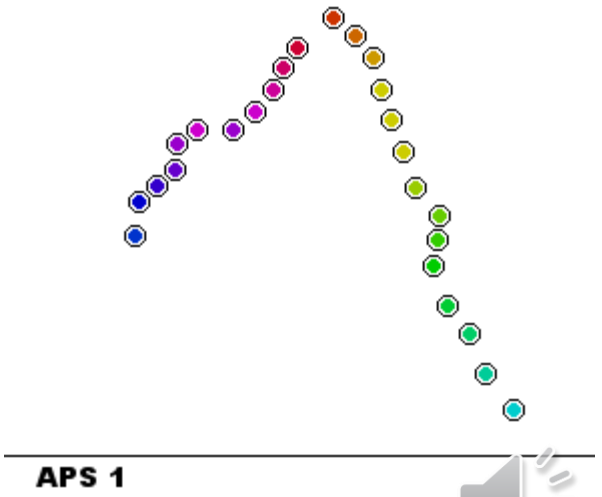
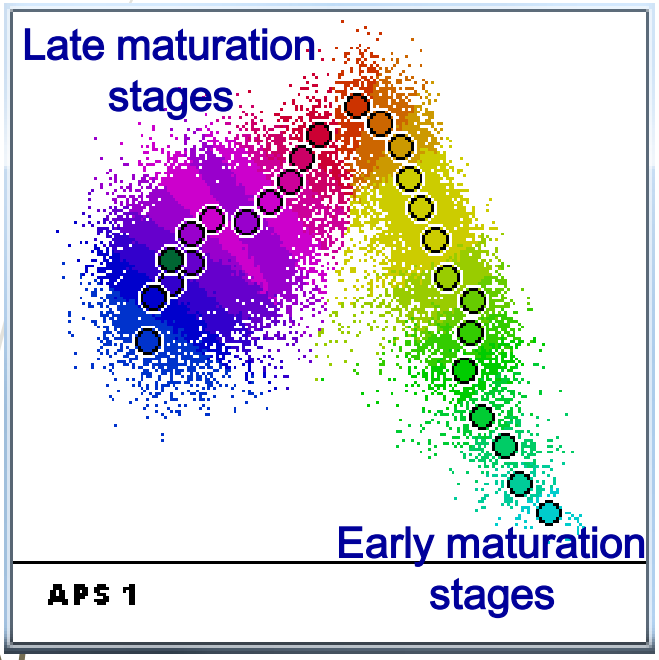




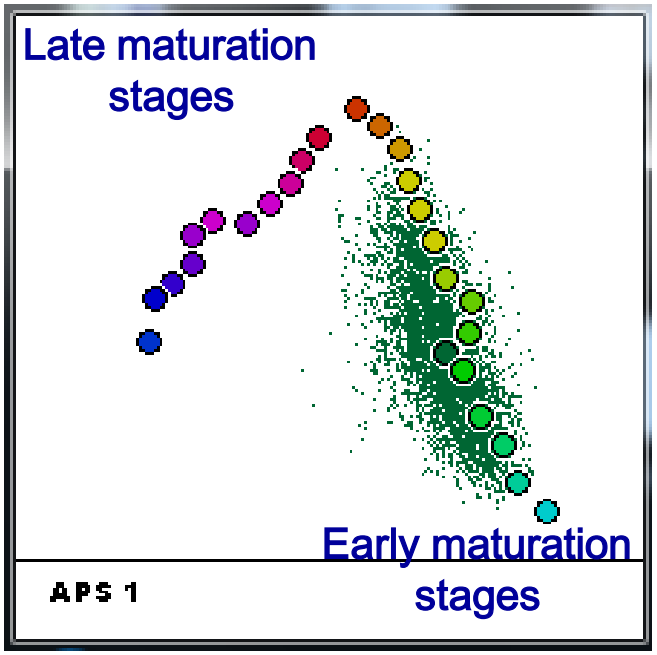
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

Diferenciación B en MO Normal vs MO regenerativa

Normal BM



Normal vs Regenerating BM



CD19 Gated B-cells (excluding PC)

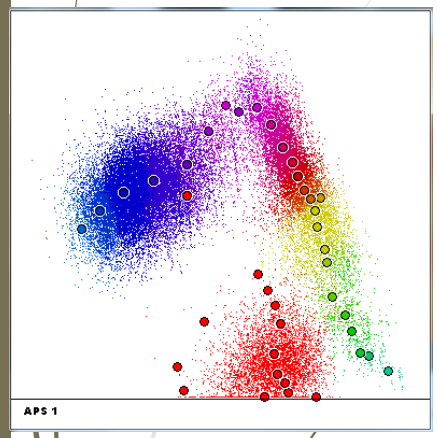
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

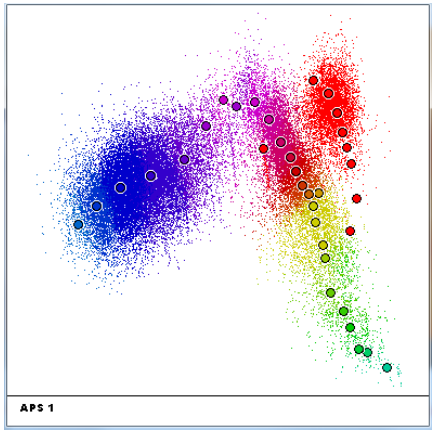
EuroFlow

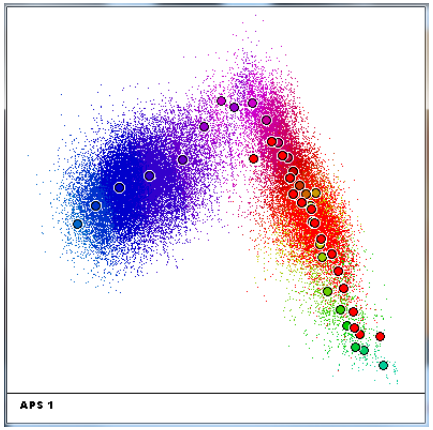
LLA vs precursores normales

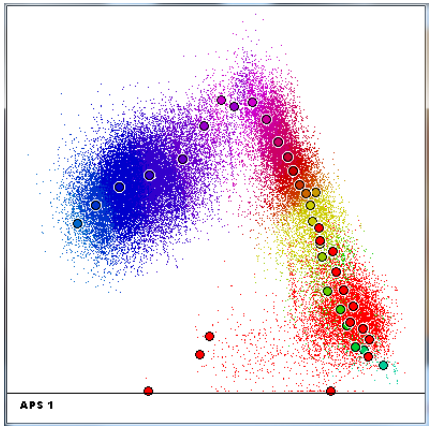
EuroFlow

APS view 1

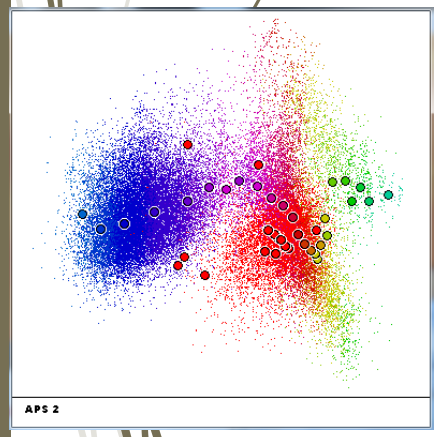


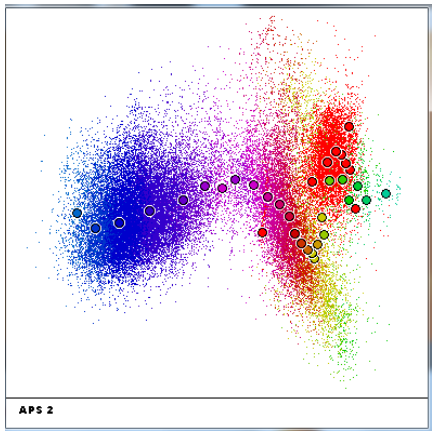


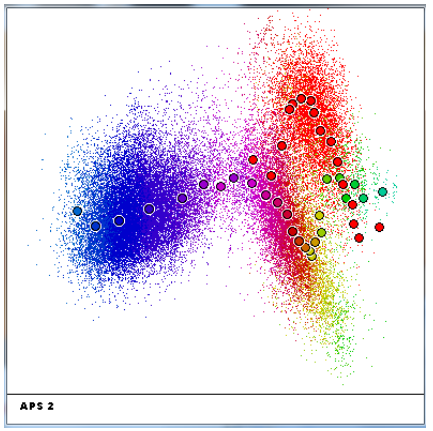


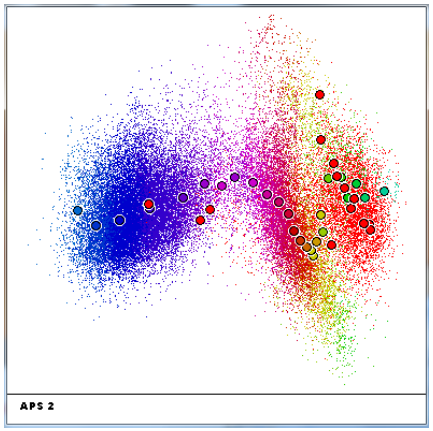


APS view 2









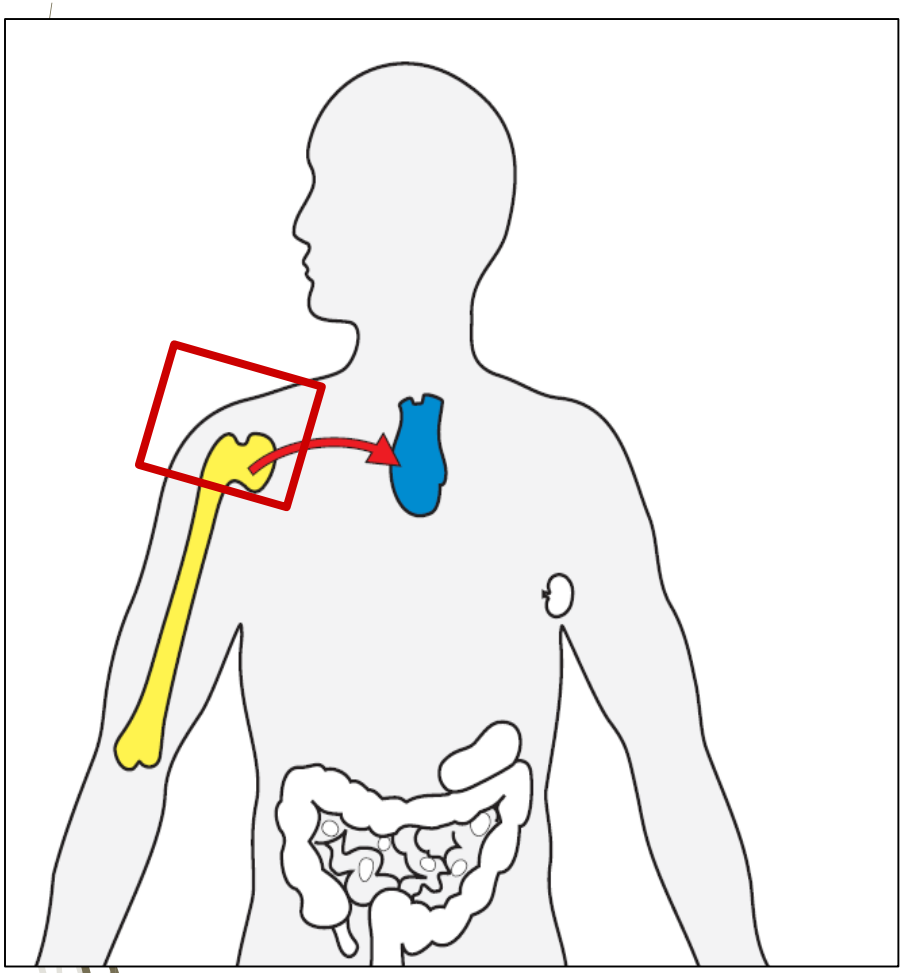
Case 1 Case 2 Case 3 Case 4

Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

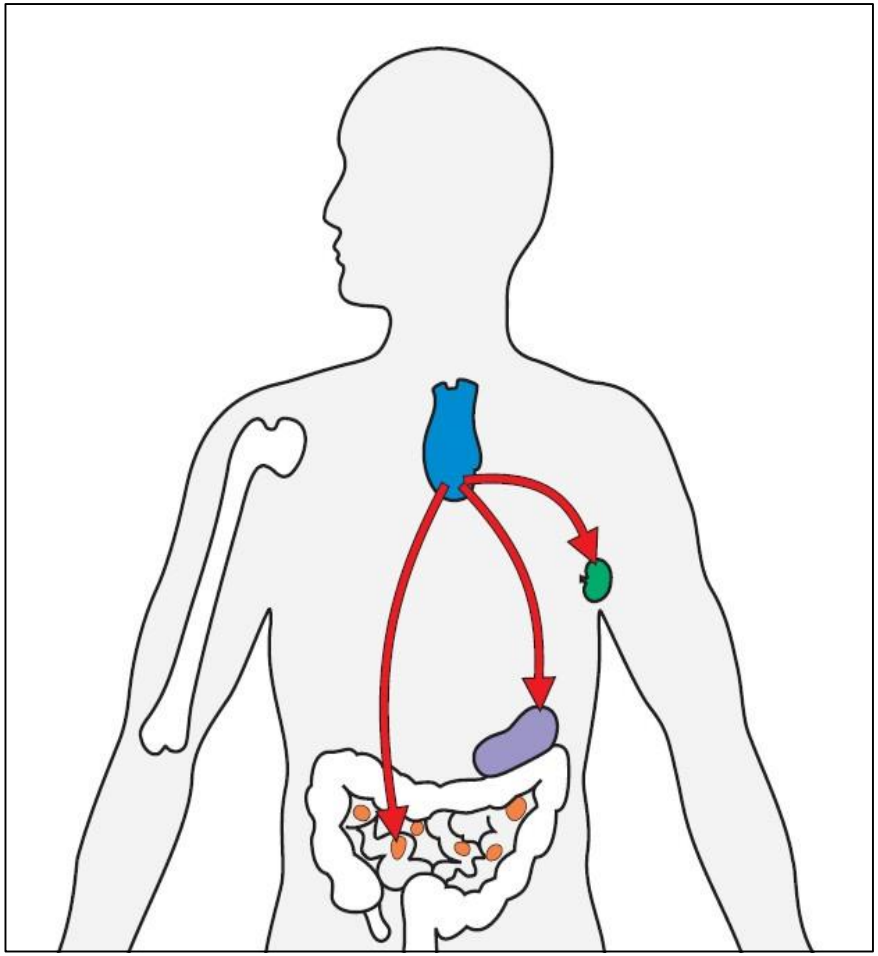
Designed by: A. Orfao & Q Lecrevisse

DIFERENCIACIÓN LINFOIDE T

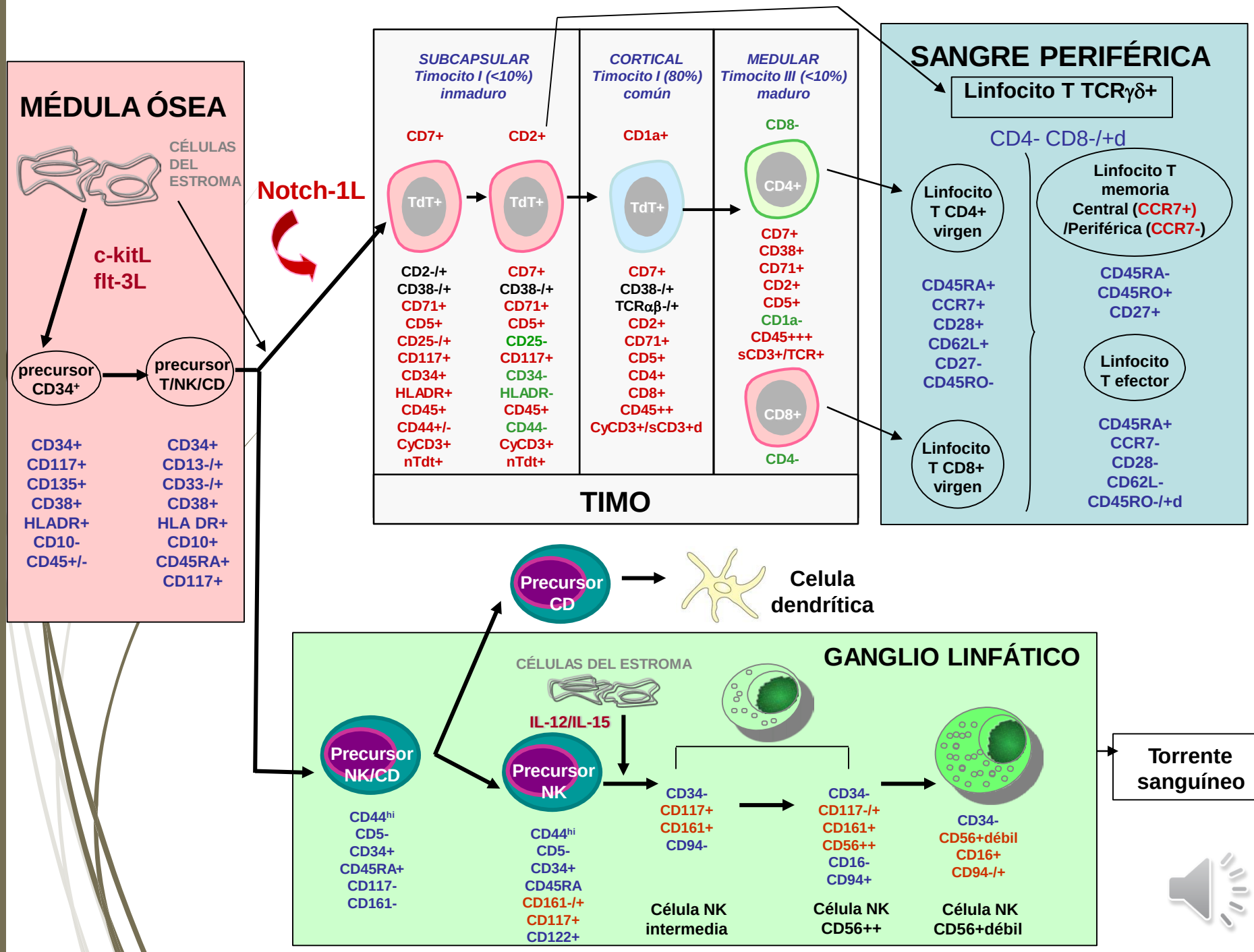
Diferenciación de linfocitos T



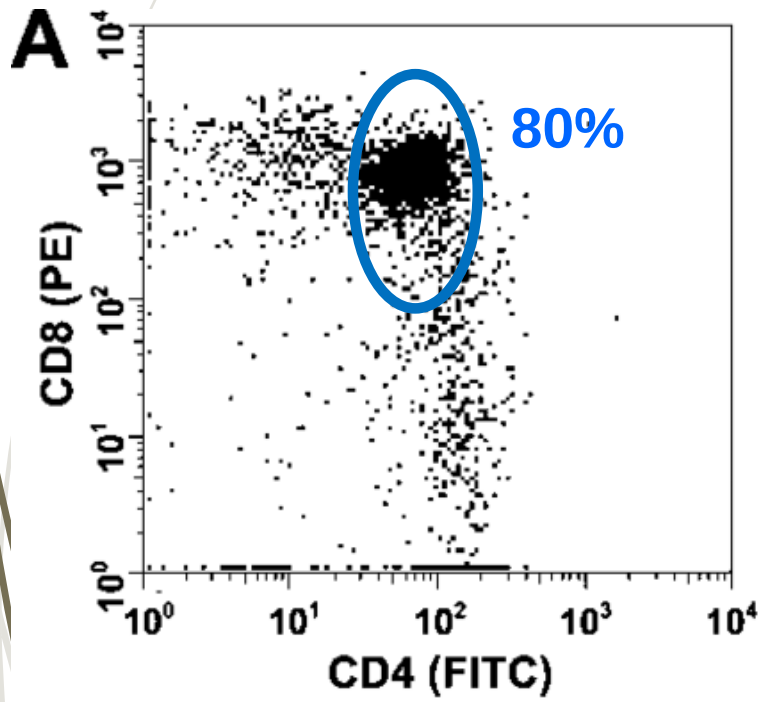
Migración de los precursores T de la médula al timo

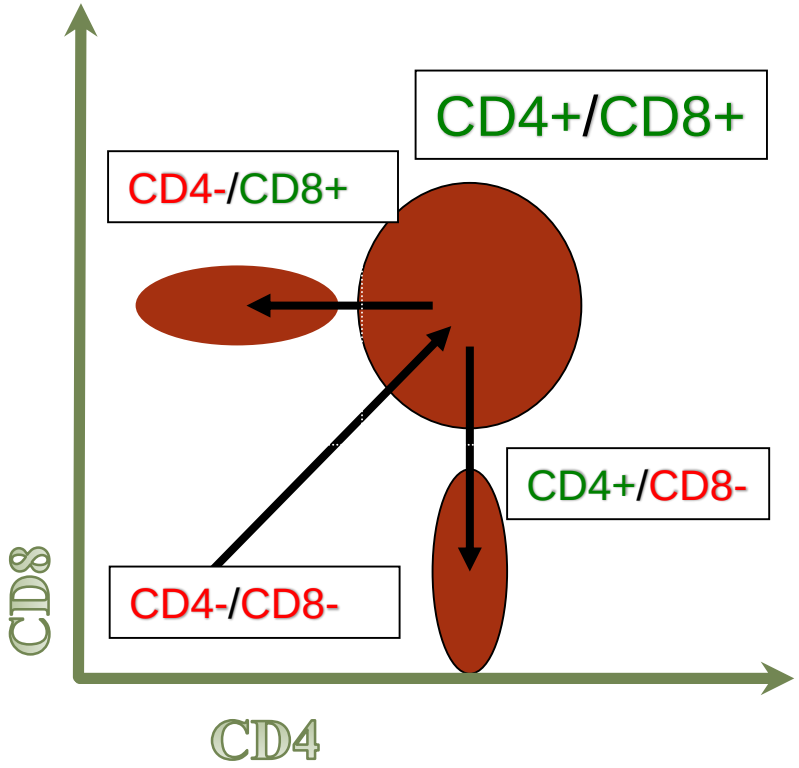


Migración de LT inmunocompetentes del timo a órganos linfoides secundarios


MÉDULA ÓSEA
CÉLULAS DEL ESTROMA
c-kitL
flt-3L
precursor CD34+
precursor T/NK/CD
CD34+ CD117+ CD135+ CD38+ HLADR+ CD10- CD45+/-
CD34+ CD13-/+ CD33-/+ CD38+ HLA DR+ CD10+ CD45RA+ CD117+
Notch-1L
SUBCAPSULAR Timocito I (<10%) inmaduro
CORTICAL Timocito I (80%) común
MEDULAR Timocito III (<10%) maduro
CD7+
CD2+
CD1a+
CD8-
TdT+
TdT+
TdT+
CD4+
CD2-/+ CD38-/+ CD71+ CD5+ CD25-/+ CD117+ CD34+ HLADR+ CD45+ CD44+/- CyCD3+ nTdt+
CD7+ CD38-/+ CD71+ CD5+ CD25- CD117+ CD34- HLADR- CD45+ CD44- CyCD3+ nTdt+
CD7+ CD38-/+ TCRαβ-/+ CD2+ CD71+ CD5+ CD4+ CD8+ CD45++ CyCD3+/sCD3+d
CD7+ CD38+ CD71+ CD2+ CD5+ CD1a- CD45+++ sCD3+/TCR+
CD8+
CD4-
TIMO
SANGRE PERIFÉRICA
Linfocito T TCRγδ+
CD4- CD8-/+d
Linfocito T CD4+ virgen
CD45RA+ CCR7+ CD28+ CD62L+ CD27- CD45RO-
Linfocito T memoria Central (CCR7+) /Periférica (CCR7-)
CD45RA- CD45RO+ CD27+
Linfocito T efector
CD45RA+ CCR7- CD28- CD62L- CD45RO-/+d
Linfocito T CD8+ virgen
Precursor CD
Celula dendrítica
GANGLIO LINFÁTICO
CÉLULAS DEL ESTROMA
IL-12/IL-15
Precursor NK/CD
Precursor NK
CD44hi CD5- CD34+ CD45RA+ CD117- CD161-
CD44hi CD5- CD34+ CD45RA CD161-/+ CD117+ CD122+
CD34- CD117+ CD161+ CD94-
Célula NK intermedia
CD34- CD117-/+ CD161+ CD56++ CD16- CD94+
Célula NK CD56++
CD34- CD56+débil CD16+ CD94-/+
Célula NK CD56+débil
Torrente sanguíneo

Expresión de CD4 y CD8 en timocitos





EGIL (II)

LLA-T

PRO-T

cCD3+d ; CD3: -

CD7+; CD45+d

PRE-T

cCD3+ ; CD3-; CD7+; CD45+d

CD2-/+ y/o CD5-/+

CORTICAL

cCD3+/++ ; CD3-/+d; CD7+;

CD45+d; CD2-/+ y/o CD5-/+;

CD1a+

LLA-T MADURA

cCD3+/++ ; CD3+d; CD7+;

CD45+d; CD2-/+; CD5-/+;

CD1a-; **CD4+ or CD8+**

CONCLUSIONES

- El conocimento de los patrones fenotípicos de maduración de las diferentes líneas es esencial para diferenciar poblaciones normales / poblaciones regenerativas /poblaciones neoplásicas independientemente de su estadío de maduración

- Se ha avanzado considerablemente en el conocimiento de los patrones fenotípicos de maduración en la hemopoyesis gracias a la citometría multiparamétrica (8 o mas colores) de alta utilidad en la valoración de la enfermedad mínima residual